CROSS - LINKED ENZYME AGGREGATES OF RECOMBINANT XYLANASE FOR CONVERSION OF HEMICELLULOSES TO XYLOOLIGOSACCHARIDES

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy *(Bioprocess Engineering)*

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MARCH 2018

I declare that this thesis entitled "*Cross-linked Enzyme Aggregates of Recombinant Xylanase for Conversion of Hemicelluloses to Xylooligosaccharides*" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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To my beloved husband (Muhamad Azraf bin Johana), mother (Hjh. Faridah Osman), brother (Prof. Dr. Sharifudin Md. Shaarani) and son (Haziq Naqiuddin).

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ABSTRACT

Hemicelluloses are heterogeneous branched polymers of sugars that exist abundantly in nature. Enzymatic hydrolysis is envisioned as a highly potential method in converting hemicelluloses into fuels and value-added chemicals. However, the use of free enzyme is hampered by low operational stability, difficulty in recovery and non-reusability, which requires for enzyme immobilization. Carrier-bound immobilization leads to utilization of high cost matrices, clogging of filters during downstream processing and presence of large amounts of non-catalytic ballast. Therefore, cross-linked enzyme aggregates (CLEA), a carrier-free technology that combines purification (precipitation) and immobilization into a single operation and does not require purified enzymes, is the solution to these problems. In this study, a recombinant xylanase (Xyl) from *Trichoderma reesei* was immobilized using three approaches: Xyl-CLEA, Xyl-CLEA-BSA (bovine serum albumin) and Xyl-CLEA-silanized maghemite. The use of ethanol as precipitant (1:9 volume ratio of enzyme to precipitant), glutaraldehyde (0.2:1 of glutaraldehyde to enzyme of 100 mM concentration) as cross-linking agent and the introduction of (3-aminopropyl) triethoxysilane (APTES) silanized maghemite (0.0075:1 of silanized maghemite to enzyme) prevailed in forming xylanase CLEAs with good enzyme activity recovery (78 %), thermal stability (50 % retained activity) and reusability (50 % retained activity). The Xyl-CLEA-silanized maghemite enhanced the activity recovery 1.66- and 1.50-fold compared to Xyl-CLEA and Xyl-CLEA-BSA, respectively. At elevated temperature of 60 °C and pHs of 3.0 and 8.0, Xyl-CLEA-silanized maghemite achieved better stability compared to the other CLEAs and free enzyme. Xyl-CLEA-silanized maghemite also successfully retained more than 50 % of its activity after 6 cycles, whereas Xyl-CLEA only retained approximately 10 % after 5 cycles. Therefore, the performance of Xyl-CLEA-silanized maghemite was further investigated by xylan hydrolysis under optimised reaction conditions. Xylooligosaccharides yield was slightly improved by 1.26- fold compared to the free enzyme. Kinetic parameters confirmed that CLEA immobilization did affect the productivity of the designed biocatalyst.

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ABSTRAK

Hemiselulosa adalah polimer bercabang heterogen yang wujud dengan sangat banyak dalam alam semulajadi. Hidrolisis enzimatik dibayangkan sebagai kaedah yang berpotensi tinggi untuk menukarkan hemiselulosa menjadi bahan api dan bahan kimia bernilai tambah. Walau bagaimanapun, penggunaan enzim bebas terhad disebabkan oleh kestabilan operasinya yang rendah, kesukaran untuk pemulihan dan ketidakbolehan guna semula, yang menyebabkan keperluan kepada proses imobilisasi enzim. Imobilisasi pembawa melibatkan penggunaan matriks yang berkos tinggi, penyumbatan penapis semasa pemprosesan hiliran dan kehadiran sejumlah besar balast bukan pemangkin. Oleh itu, agregat enzim terpaut silang (CLEA), teknologi bebas pembawa yang menggabungkan pemurnian (pemendakan) dan imobilisasi ke dalam satu operasi dan tidak memerlukan enzim tulen, adalah penyelesaian kepada masalah ini. Dalam kajian ini, *xylanase* rekombinan (Xyl) dari *Trichoderma reesei* diimobilisasi menggunakan tiga pendekatan: Xyl-CLEA, Xyl-CLEA-BSA *(bovine serum albumin)* dan Xyl-CLEA-*silanized maghemite*. Penggunaan etanol sebagai pemendak (1:9 nisbah isipadu enzim kepada pemendak), glutaraldehid (0.2:1 glutaraldehid kepada enzim berkepekatan 100 mM) sebagai agen pemaut silang dan pengenalan *(3-aminopropyl) triethoxysilane* (APTES) *silanized maghemite* (0.0075: 1 *silanized maghemite* kepada enzim) berjaya membentuk *xylanase* CLEA dengan pemulihan aktiviti enzim yang baik (78 %), kestabilan terma (50 % aktiviti tersimpan) dan kebolehan guna semula (50 % aktiviti tersimpan). Xyl-CLEA-*silanized maghemite* meningkatkan pemulihan aktiviti 1.66- dan 1.50- kali ganda berbanding dengan Xyl-CLEA dan Xyl-CLEA-BSA, masing-masing. Pada suhu tinggi 60 °C dan pH 3.0 dan 8.0, Xyl-CLEA-*silanized maghemite* mencapai kestabilan yang lebih baik berbanding dengan CLEA yang lain dan enzim bebas. Xyl-CLEA-*silanized maghemite* juga berjaya mengekalkan lebih daripada 50 % aktiviti selepas 6 kitaran, manakala Xyl-CLEA hanya mengekalkan kira-kira 10 % selepas 5 kitaran. Oleh itu, prestasi Xyl-CLEA-*silanized maghemite* dikaji selanjutnya melalui hidrolisis xilan di bawah keadaan tindak balas yang dioptimumkan. Hasil xilooligosakarida meningkat sebanyak 1.26- kali ganda berbanding dengan enzim bebas. Parameter kinetik mengesahkan bahawa imobilisasi CLEA mempengaruhi produktiviti biomangkin yang direka.

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LIST OF ABBREVIATIONS

A	-	Absorbance
ANOVA	-	Analysis of variance
ATCC	-	American type culture collection
B	-	Blank / buffer only
BMGY	-	Buffered glycerol-complex medium
BMMY	-	Buffered methanol-complex medium
BSA	-	Bovine serum albumin
CCD	-	Central composite design
dH_2O	-	Distilled water
DNS	-	3, 5-Dinitrosalicylic acid
EC	-	Enzyme control
e.g.	-	“for example”
etc.	-	et cetera / “and so forth”
g	-	gram
g/L	-	gram per liter
h	-	hour
HCl	-	Hydrochloric acid
HPLC	-	High performance liquid chromatography
i.e.	-	“that is”
kDa	-	kilo Dalton
L	-	Liter
M	-	Molar
min	-	minute
mL	-	milliliter
mm	-	millimete

mM	-	milimolar
MWCO	-	Molecular weight cut off
NaOH	-	Sodium hydroxide
nm	-	nanometer
OD	-	Optical density
OFAT	-	One-factor-at-a time
pI	-	Isoelectric point
PMSF	-	Phenylmethylsulfonyl fluoride
PEG	-	Polyethylene glycol
RBB	-	Remazol brilliant blue
RI	-	Refractive index
Rpm	-	revolutions per minute
RSM	-	Response surface methodology
SC	-	Substrate control
SD	-	Standard deviation
SDS PAGE	-	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
T	-	Temperature
TRS	-	Total reducing sugar
U	-	Unit enzyme
UV	-	Ultra violet
V	-	Velocity
v/v	-	Volume per volume
w/w	-	Weight per weight
w/v	-	Weight per volume
X1	-	Xylose
X2	-	Xylobiose
X3	-	Xylotriose
X4	-	Xylotetraose
X5	-	Xylopentaose
X6	-	Xylohexaose
XOS	-	Xylooligosaccharides
Xyn2	-	Xylanase2
YPD	-	Yeast extract peptone dextrose

LIST OF SYMBOLS

˚C	-	Degree Celsius
°F	-	Degree Fahrenheit
%	-	Percentage
α	-	alpha
β	-	beta
γ	-	gamma
µL	-	microlitre
10X D	-	Ten times dilution
µg/mL	-	microgram per litre
1X	-	One time
µmol	-	micromole
5X	-	Five times
3D	-	Three dimensions
™	-	Trademark symbol
~	-	Approximate value

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